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Comparison of Heat and Hydrostatic Pressure Shocks to Induce Triploidy in Steelhead Trout (*Oncorhynchus mykiss*)

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COMPARISON OF HEAT AND HYDROSTATIC PRESSURE SHOCKS TO INDUCE TRIPLOIDY IN STEELHEAD TROUT (Oncorhynchus mykiss)

by

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ABSTRACT

Guoxiong, C., I. I. Solar, and E. M. Donaldson. 1989. Comparison of heat and hydrostatic pressure shocks to induce triploidy in steelhead trout (Oncorhynchus mykiss). Can. Tech. Rep. Fish. Aquat. Sci. No. 1718: 11 p.

Triploid female salmonids are known to be sterile and thus may offer certain advantages for aquaculture. This study was conducted to compare various temperature and pressure shocks for the induction of triploidy in steelhead trout, an anadromous form of Oncorhynchus mykiss.

Thermal or hydrostatic pressure shocks were administered 25 min post-fertilization to groups of steelhead trout eggs. Thermal shocks ranged from 26 to 36°C, lasting from 1.25 to 20 min. Pressure shocks ranged from 5.5 to 8.3 x 10^4 kPa, lasting from 2 to 6 min. Triploid induction was evaluated by measurement of the major axis of erythrocyte nuclei. Triploid rates of 100% were produced by heat shock at 26°C for 20 min and by pressure shock of 7.6 x 10^4 kPa for 6 min. Survival, however, was inversely correlated to the intensity of the treatments, thus triploid yields, calculated as the product of the triploid rate and the numbers surviving to hatch, expressed as percentage of the number of eggs originally present in the groups, were best when a heat shock of 26°C for 10 min or hydrostatic pressure treatment at 6.9 x 10^4 kPa for 6 min was applied (50.3% and 49.9%, respectively).

Key words: triploidy, heat shock, pressure shock, sterility, steelhead trout

RÉSUMÉ

Guoxiong, C., I. I. Solar, and E. M. Donaldson. 1989. Comparison of heat and hydrostatic pressure shocks to induce triploidy in steelhead trout (Oncorhynchus mykiss). Can. Tech. Rep. Fish. Aquat. Sci. No. 1718: 11 p.

Les femelles triploïdes des salmonidés sont stériles et représentent donc certains avantages pour l'aquiculture. La présente étude visait à comparer l'efficacité de divers chocs de température et de pression pour l'induction de la triploïdie chez la truite arc-en-ciel, une forme anadrome d'Oncorhynchus mykiss.

Les chocs thermiques ou les chocs par application de pression ont été administrés à des groupes d'oeufs de truites arc-en-ciel 25 minutes après la fécondation. Des chocs thermiques, variant de 26 à 36°C, ont été appliqués durant 1,25 à 20 minutes et des chocs par application de pression, variant de 5,5 à 8,3 x 10^4 kPa, ont été appliqués durant 2 à 6 minutes. On a évalué l'induction de la triploïdie en mesurant l'axe principal du noyau des érythrocytes. On a obtenu des taux de triploïdie de 100% avec des chocs thermiques de 26°C durant 20 minutes et avec des chocs par application de pression de 7,6 x 10^4 kPa durant 6 minutes. Cependant, la survie a été inversement proportionnelle à l'intensité des traitements. Le rendement en sujets triploïdes, c'est-à-dire le produit du taux de triploïdie et du nombre de sujets ayant survécu jusqu'à l'éclosion, exprimé sous forme de pourcentage du nombre d'oeufs présents dans les groupes de départ, a été optimal dans les conditions suivantes: choc thermique de 26°C pendant 10 minutes ou choc par application de pression de 6,9 x 10^4 kPa pendant 6 minutes (50,3 % et 49,9 % respectivement).

Mots-clés: triploïdie, choc thermique, choc par application de pression, stérilité, truite arc-en-ciel

INTRODUCTION

Induction of triploidy is one of the promising controlled reproduction biotechnologies applicable to aquaculture (Donaldson and Hunter, 1982; Thorgaard, 1983; Donaldson, 1986), and has received considerable attention from researchers.

Triploidy has been shown to cause sterility in salmonids which can be of benefit under culture conditions (Wolters, et al., 1982; Benfey and Sutterlin, 1984; Lincoln and Scott, 1983, 1984; Solar, et al., 1984; Benfey et al., 1987; Donaldson and Benfey, 1987). Several methods have been proposed for triploid production in fish, including crosses of tetraploid with diploid brookstock (Chourrout, 1982), and chemical-induced dispermy (Ueda et al., 1986; Ueda et al., 1988). However, most attempts have involved the direct induction of triploidy by blocking the extrusion of the second polar body. In salmonids, this has been achieved by means of thermal shock (Chourrout, 1980; Thorgaard et al., 1981; Benfey and Sutterlin, 1984; Johnstone, 1985), hydrostatic pressure shock (Benfey and Sutterlin, 1984; Benfey et al., 1988), or chemical and gas pressure treatments (Shelton et al., 1986) under a variety of regimes (i.e. varying temperature, pressure, dosage, treatment duration, and time post-fertilization). These studies have shown that significant proportions, even up to 100% triploids could be induced on an experimental scale.

In the context of widespread application of triploid induction techniques in intensive aquaculture some problems still remain such as the establishment of standard optimal treatment conditions for a high degree of artificial triploidisation in individual species of economic significance, using direct thermal, hydrostatic pressure or chemical shocks or indirect techniques involving diploid spermatozoa.

The present paper describes the results of a study on induced triploidy in the steelhead trout (Oncorhynchus mykiss), a sea-going rainbow trout, which compared a particularly wide range of heat and hydrostatic pressure shocks on a single pool of eggs and which was designed to establish an optimal treatment regime for the species.

MATERIALS AND METHODS

Handling of gametes and fertilization procedures

Ova obtained from two females and sperm collected from two males were pooled, divided into two groups(for pressure and heat shocks, respectively) and stored in a cooler at 4.5 - 5.5°C. Pressure shocks (PS) were applied on the same day starting within one hour after gamete collection. Heat shocks (HS) were applied the following day starting at 24 h

after spawning. The gametes were stored overnight in the cooler in oxygen filled plastic bags.

The same fertilization and pre-treatment procedures were used for PS and HS. Replicated groups of eggs (90 ± 15 eggs) were inseminated with 0.25 ml of sperm in a 50 ml plastic beaker, water activated after 10-20 sec, rinsed after 30 sec and incubated at 7.5°C for 25 min prior to treatment.

Hydrostatic pressure and thermal shocks

Pressure shocks were applied using a Carver laboratory press Model C12 at pressures of 5.5, 6.2, 6.9, 7.6 or 8.3 x 10^4 KPa for 2, 4 or 6 min. Two sets of triplicate control groups were placed in the pressure chamber for the same durations, but no pressure was applied. The thermal shocks were applied by placing the eggs in perforated plastic cylinders (7 cm diameter, 7 cm height) with mesh bottoms. Five groups (and their replicates) were immersed simultaneously in a thermoregulated recirculating water bath (JULABO 36B) at temperatures of 26, 28, 30, 32, 34 and 36°C. Each set of replicates was removed after 1.25, 2.5, 5, 10 and 20 min and cooled with ambient temperature water (approx. 8.0°C) before transferring to the incubators. Replicated control groups were immersed in the water bath for 10 min, but ambient water temperature was used. Another 2 sets of controls received no treatment at all.

Incubation and hatching

After treatment the groups were placed in 16-compartment plexiglass units with 1 mm plastic mesh bottoms inserted in Heath incubation trays supplied with 10 lpm flowing well water at 11°C mean temperature (range 7.5 - 12.1°C). Incubation was conducted in darkness and until the eyed-stage 80 ml of 1% malachite green solution was added to the top tray every four days. Fertilization and survival rates to the eyed-stage were determined by observation of embryonic development in dead eggs removed and placed in a clearing solution (5% Acetic acid, 9% NaCl). Afterwards, dead embryos and/or larvae were removed periodically and quantities recorded. Median hatch took place on day 34 (350.2°C d). Swim-up occurred on day 65 (667.7 °C d).

Ploidy determination

Ploidy was determined indirectly by measurement of the major axis of red blood cell nuclei, a widely accepted procedure for rapid determination of ploidy (Cimino, 1973; Wolters et al. 1982; Benfey et al., 1984). Blood smears were prepared from up to 10 fish per group according with the procedures described by Johnstone and Lincoln (1986). Mean and standard deviation of erythrocyte size was calculated by measuring 10 to 20 cells per fish.

Survival was calculated as the percentage of the treated eggs that hatched. Triploid rate is the percentage of alevins among the fish sampled

that had a mean erythrocyte size near 50% larger than diploid controls. Triploid yield was calculated as the product of the triploid rate and the numbers surviving to hatch, expressed as the number of eggs originally present in the group.

RESULTS AND DISCUSSION

Measurement of the major axis in erythrocyte nuclei revealed that normal diploid fish had a mean 6.07 ± 0.82 u while triploid fish had a mean of 8.78 ± 0.64 u; the major nuclear axis of triploids being approximately 45% larger than the axis of diploids. Fish with red blood cell size typical of that of triploids were also found among 2 control groups. The results obtained in the various treatments and control procedures are summarized in Tables 1 and 2, (Figs. 1 to 4). High triploid rates were achieved with the more intense treatments (either higher temperatures and pressures or longer durations at the same level of temperature and pressure). Survival rates were, however, inversely proportional to triploid rates, resulting in triploid yields that were best at 6.9×10^4 kPa for 6 min (49.9%) and at 26°C for 10 min (50.3%).

Most of the mortalities during embryonic development occurred around the eyed-egg stage. Highest intensity temperature shocks caused complete mortalities. By comparison even the highest intensity pressure shock (8.3×10^4 kPa) allowed relatively good survival (20 to 38% over a duration of 2-6 min).

Survival rates among controls which received handling similar to experimental groups were lower than in unhandled controls. Within the pressure shock experiment, the survival of controls decreased slightly as the period the eggs remained in the pressure chamber was increased.

This study is, to our knowledge, the first report on the application of two of the most widely used techniques for polyploid induction, heat and pressure shocks, on the same pool of eggs from a salmonid species, the steelhead trout. This has allowed us to compare directly the efficiency of both methods without the constraints observed in previous reported studies that have shown inconsistent results with either technique usually attributed to different degrees of egg ripeness at the time of the trials. In the present study, we utilized a pool of gametes from two females and two males and the treatments using both techniques were applied to similar aliquots one day apart.

Both procedures produced similar results as far as triploid rates induced. Although survival rates were in general very similar for both procedures, there was a higher incidence of deformities among the heat shock treated groups, compared with the pressure treated groups. This is in agreement with observations by Gillespie and Armstrong (1979) comparing heat and pressure to induce triploidy in salamanders.

The presence of one triploid individual in each of 2 control groups was unexpected. Previous reports, however, (Cuellar and Uyeno, 1978; Allen and Stanley, 1978; Thorgaard and Gall, 1979; Ueda et al., 1988) clearly indicate that spontaneous triploids are not uncommon in salmonids.

Neither of the best two triploid yields obtained in this study (50.3 and 49.9%) were the result of particularly high triploid rates (70 and 85%, respectively). The third best (48.9%) however, resulted of the combination of 100% triploid induction and close to 50% survival in the groups submitted to 26°C for 20 min. These results suggest that if the production of an all triploid stock is the desired goal, the shock at 26°C for 20 min would be the optimal treatment for this species.

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REFERENCES

Allen, S. K., and J. G. Stanley. 1978. Reproductive sterility in polyploid brook trout, Salvelinus fontinalis. Trans. Am. Fish Soc., 107(3): 473-478.

Benfey, T. J., and A. M. Sutterlin. 1984. Triploidy induced by heat shock and hydrostatic pressure in landlocked Atlantic salmon (Salmo salar L.). Aquaculture. 36: 359-367.

Benfey, T. J., and A. M. Sutterlin. 1984. Growth and gonadal development in triploid landlocked Atlantic salmon (Salmo salar). Can. J. Fish. Aquat. Sci. 41: 1386-1387.

Benfey, T. J., A. M. Sutterlin, and R. J. Thompson. 1984. Use of erythrocyte measurement to identify triploid salmonids. Can. J. Fish. Aquat. Sci. 41: 980-984.

Benfey, T. J., I. I. Solar, and E. M. Donaldson. 1987. The reproductive physiology of triploid Pacific salmonids. p. 128 In: Marine Sciences Research Lab. Memorial University of Newfoundland, Proceedings of the Third International Symposium on the Reproductive Physiology of Fish, St. John's, Newfoundland, Canada, Aug. 2-7, 1987.

Benfey, T. J., P. G. Bosa, N. L. Richardson, and E. M. Donaldson. 1988. Effectiveness of a commercial-scale pressure shocking device for producing triploid salmonids. Aquacult. Eng. 7: 147-154.

Chourrout, D. 1980. Thermal induction of diploid gynogenesis and triploidy in the eggs of the rainbow trout (Salmo gairdneri Richardson). Reprod. Nutr. Dev. 20: 733.

Chourrout, D. 1982. Tetraploidy induced by heat shocks in the rainbow trout (Salmo gairdneri R.). Reprod. Nutr. Dev. 22(3): 569-574.

Cimino, M. C. 1973. Karyotypes and erythrocyte sizes of some diploid and triploid fishes of the genus Poeciliopsis. J. Fish. Res. Board Can. 30: 1736-1737.

Cuellar, O. and T. Uyeno. 1972. Triploidy in rainbow trout. Cytogenetics. 11: 508-515.

Donaldson, E. M. 1986. The integrated development and application of controlled reproduction techniques in Pacific salmonid aquaculture. Fish Physiol. Biochem. 2(1-4): 9-24.

Donaldson, E. M., and G. A. Hunter. 1982. Sex control in fish with particular reference to salmonids. Can. J. Fish. Aquat. Sci. 39: 99-110.

Donaldson, E. M., and T. J. Benfey. 1987. Current status of sex manipulation. p.108-119 In: Marine Sciences Research Lab. Memorial University of Newfoundland, Proceedings of the third international symposium on the reproductive physiology of fish, St. Newfoundland, Canada Aug. 2-7, 1987.

Gillespie, L. L., and J. B. Armstrong. 1979. Induction of triploidy and gynogenetic diploid axolotis (Ambystoma mexicanum) by hydrostatic pressure. J. Exp. Zool., 120: 117-122.

Johnstone, R. 1985. Induction of triploidy in Atlantic salmon by heat shock. Aquaculture. 49: 133-139.

Johnstone, R., and R. R. Lincoln. 1986. Ploidy estimation using erythrocytes from formalin-fixed salmonid fry. Aquaculture. 55: 145-148.

Lincoln, R. F., and A. P. Scott. 1983. Production of all-female triploid rainbow trout. Aquaculture. 30: 375-380.

Lincoln, R. F., and A. P. Scott. 1984. Sexual maturation in triploid rainbow trout, Salmo gairdneri Richardson. J. Fish Biol. 25: 385-392.

Shelton, C. J., A. G. Macdonald, and R. Johnstone. 1986. Induction of triploidy in rainbow trout using nitrous oxide. Aquaculture. 58: 155-159.

Solar, I. I., E. M. Donaldson, and G. A. Hunter. 1984. Induction of triploidy in rainbow trout (Salmo gairdneri Richardson) by heat shock, and investigation of early growth. Aquaculture. 42: 57-67.

Thorgaard, G. H. 1983. Chromosome sex manipulation and sex control in fish, p. 405-434. In: W. A. Hoar, D. J. Randall and E. M. Donaldson (Eds.), Fish Physiology. Vol. 9. Academic Press, New York, NY.

Thorgaard, G. H., M. E. Jazwin, and A. R. Stier. 1981. Polyploidy induced by heat shock in rainbow trout. Trans. Am. Fish. Soc. 110: 546-550.

Ueda, T., M. Kobayashi, and R. Sato. 1986. Triploid rainbow trouts induced by polyethylene glycol. Proc. Japan Acad. 62, Ser. B. p. 161.

Ueda, T., R. Sato, and J. Kobayashi. 1988. Triploid rainbow trout induced by high-pH high-calcium. Nippon Suisan Gakkaishi. 54: 2045.

Wolters, W. R., C. L. Chrisman, and G. S. Libey. 1982. Erythrocyte nuclear measurements of diploid and triploid channel catfish, Ictalurus punctatus (Rafinesque). J. Fish Biol. 20: 253-258.

TABLE 1. Mean triploid yield of heat shocked steelhead trout eggs.

Duration (min)	Temperature °C 26	28	30	32	34	36
1.25	12.4	11.0	0.0	3.0	0.0	5.9
2.50	21.6	22.4	17.1	11.5	1.2	0.0
5.0	28.8	29.8	27.9	0.0	0.0	0.0
10.0	50.3	26.2	0.0	0.0	0.0	0.0
20.0	48.9	26.6	0.0	0.0	0.0	0.0
control (a)	0.0					
control (b)	5.2					

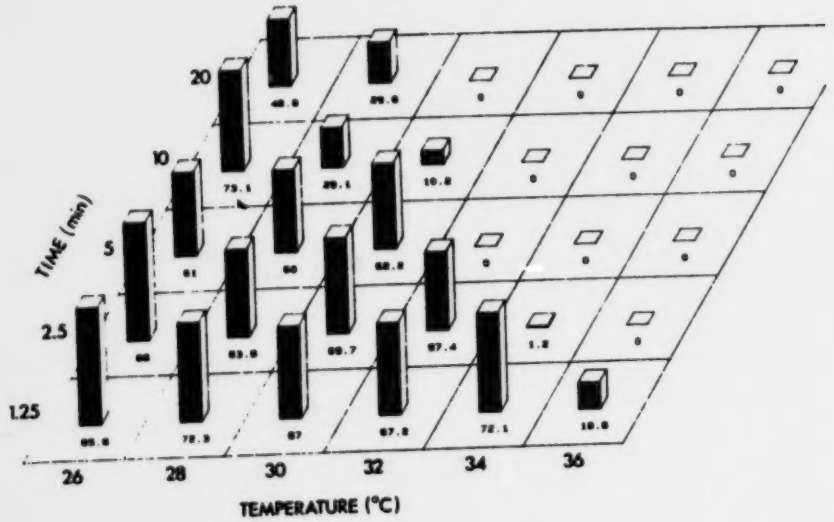
a) control with same handling procedures as experimental group, but without heat shock; three replicates.
b) unhandled controls; three replicates.

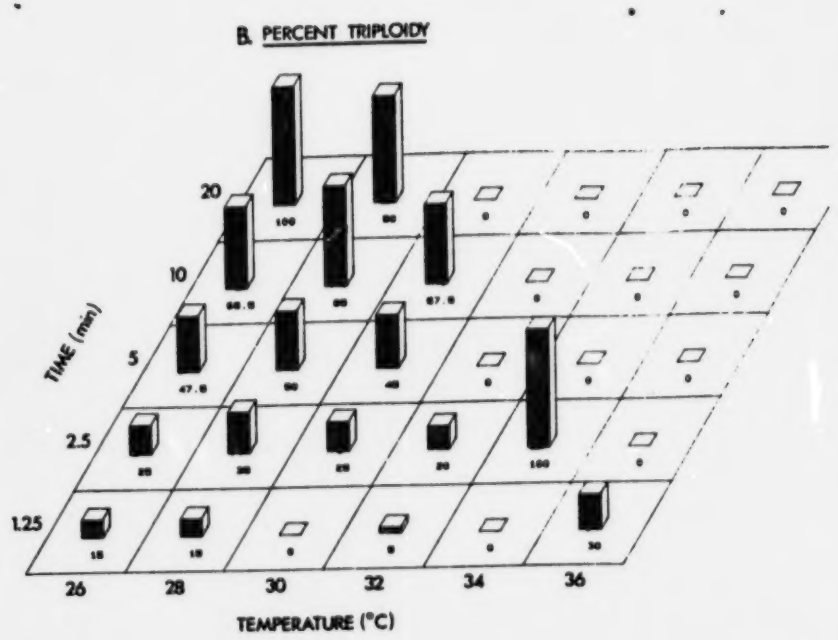
TABLE 2. Mean triploid yield of pressure shocked steelhead trout eggs.

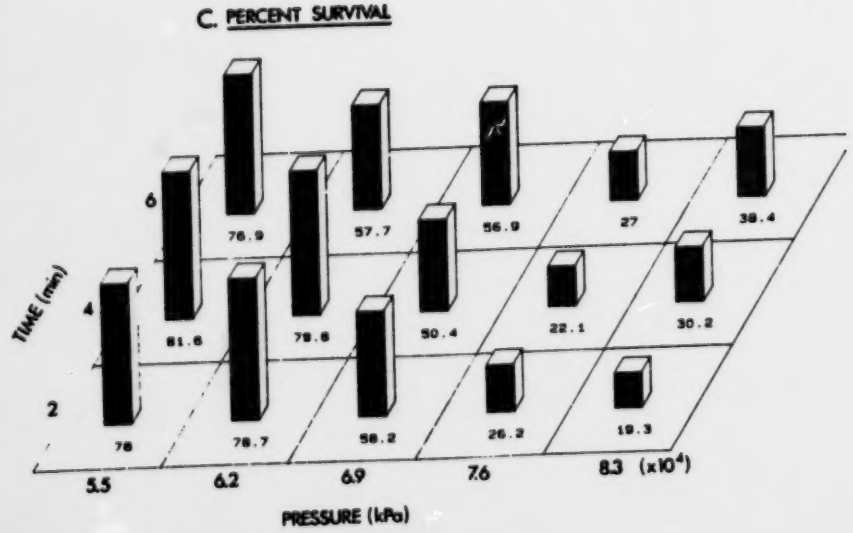
Duration (min)	Hydrostatic pressure (x 10^4 KPa) 5.5	6.2	6.9	7.6	8.3	control[a]
2	9.3	3.9	0.0	3.3	13.7	0.0
4	0.0	3.7	20.2	18.8	28.1	0.0
6	11.5	20.1	49.9	27.0	30.2	0.0
control (b)	0.0 (82.0 - 0.0)					

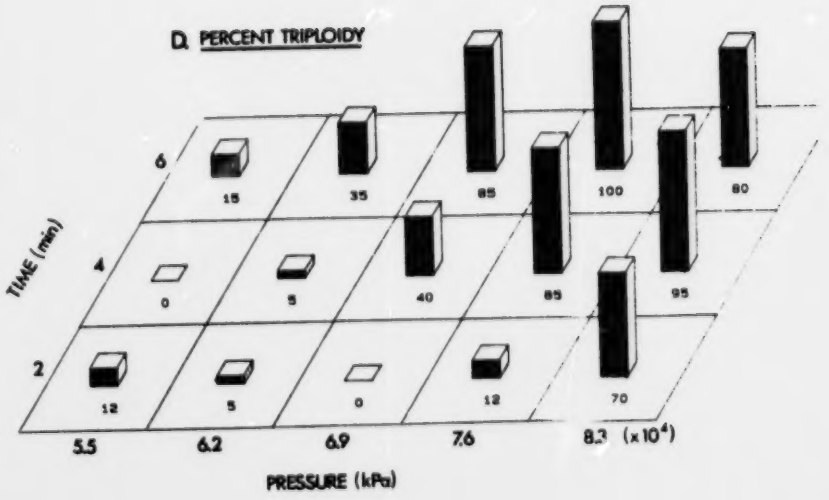
a) control with same handling procedures as experimental group, but without pressure shock.
b) unhandled controls

Fig. 1. Percent survival and percent triploidy induced in fertilized steelhead eggs by heat shock (A and B) or by hydrostatic pressure shock (C and D) in relation to treatment intensity in degree celcius (°C) or kilopascos (kPa) and duration (minutes).


A. PERCENT SURVIVAL
TIME (min)
1.25
2.5
5
10
20
26
28
30
32
34
36
TEMPERATURE (°C)


B. PERCENT TRIPLOIDY
TIME (min)
1.25
2.5
5
10
20
26
28
30
32
34
36
TEMPERATURE (°C)


C. PERCENT SURVIVAL
TIME (min)
2
4
6
5.5
6.2
6.9
7.6
8.3 (x10⁴)
PRESSURE (kPa)


D. PERCENT TRIPLOIDY
TIME (min)
2
4
6
5.5
6.2
6.9
7.6
8.3 (x10⁴)
PRESSURE (kPa)